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Research on the preparation and adsorption properties of melamine molecularly imprinted polymers

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Abstract. New magnetic melamine imprinted polymer has been prepared with magnetic graphene oxide (GO) as the carrier, melamine (MEL) as the template molecule, methacrylic acid (MAA) as functional monomer and ethylene glycol dimethacrylate (EGDMA) as crosslinking agent. Such magnetic imprinted polymer is characterized and analyzed by using scanning electron microscopy (SEM), transmission electron microscopy (TEM), differential thermal analysis (TG) and vibrating sample magnetometer (VSM). Results indicate that magnetic imprinted polymer is prepared successfully on surface of GO. After testing the adsorptive property of such imprinted polymer with combination of analysis technology of high performance liquid chromatography (HPLC), the results indicate that such magnetic imprinted polymer shows adsorption specificity to melamine. Combined with magnetic solid phase extraction and liquid chromatography detection technology, separation, enrichment and test of melamine in milk sample have been realized.

Key words. Melamine, Molecular imprinting, Polymer, Adsorptive property, Milk sample.

1. Introduction

Food is the material basis for human survival and development. However, with the development of science and technology as well as social progress, food safety problems occur more and more frequently, more and more serious. In recent years, Suyue Yihong, carcinogenic rice, melamine, seriously exceeded pesticide residues in fruits and vegetables and other food safety incidents came one after another so that food safety testing is extremely urgent.

In order to ensure food safety, we shall ensure the reliability and accuracy of test results strictly. At present there are many methods for food safety testing. However,

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in these common methods, such as chemical method, immunization and biological test method, samples are needed to be pretreated complexly. If sample composition is very complicated and when the a large number of samples are tested, large testing instruments and professional operators are also needed in these method as mentioned above, which is costly and time-consuming as well as laborious. In this context, the simpler, time-saving, cost-effective new testing technology and sample pretreatment method are needed to be developed urgently, in which, molecular imprinting technology with rapid development is a kind of new testing technology. Such technology has the advantages of simple operation, accuracy and reliability, which is widely used and applicable to analysis and test of industrial raw material additives and pesticide residue in food.

2. Experiment

2.1. Reagents and instruments

Methacrylic acid (MAA), ethylene dimethacrylate (EDMA), benzoguanamine and Sodium 1-octanesulfonate are chromatographically pure, purchased from Beijing J&K Science and Technology Co., Ltd. Melamine (99%, Tianjin Bodi Chemical Co., Ltd.), acetonitrile, methanol are chromatographically pure, purchased from Tianjin Fuchen Chemical Reagents Factory. Anhydrous magnesium sulfate, citric acid, azodiisobutyronitrile (AIBN), sodium hydroxide, glacial acetic acid, isooctane, ammonium hydroxide, methanol and toluene are analytically pure, purchased from Tianjin Fengchuan Chemical Reagent Technologies Co., Ltd. PRO-KIDO older infant formula milk powder is purchased from Inner Mongolia Yili Industrial Group Co., Ltd. Redistilled water is used in this experiment.

Aailent 1100 HPLC, TENSOR 27 infrared spectrometer (BRUKER, Germany), Quanta 200 SEM (FEI, Hillsboro, Oregon, USA), PoreMaster 33 mercury injection apparatus (Quantachrome, USA>), solid phase extraction device, termovap sample concentrator (Supelco, USA), LTV 2100 dual-beam UV - visible spectrophotometer (Beijing Ruili Analytical Instrument Co., Ltd.), TG-16 high speed centrifuge (Gongyi Yuhua Instrument Co., Ltd.), TWC L-D homothermic magnetic stirrer (Henan Aibote Technology Development Co., Ltd) and XK 96-B rapid mixing device (Xinkang Medical Equipment Co., Ltd).

2.2. Preparation of imprinted polymer

GO is prepared with improved Hummers method. 1.0g graphite and 1.2g potassium nitrate are fully grinded to be mixed evenly. Then 46mL concentrated sulfuric acid is added into it with slowly stirring in ice-water bath at controlled temperature of 4°, which is followed by 6g potassium permanganate. Ice-water bath is removed after adding potassium permanganate and it is kept warm for 1h at controlled temperature of 35°. Then 90mL water is added slowly to rise the temperature to above 98°, with keeping it warm for 30min. Following 200mL deionized water, 6mL 30% H_2O_2 solution is added slowly drop by drop. Reaction is completed when color so-

lution becomes the bright yellow. After suction filtration, product is washed with deionized water firstly to be put into dryer for drying in 36h. Then the bulk solid product is obtained, which is grinded for obtaining GO powder for standby, as shown in Fig. 1.

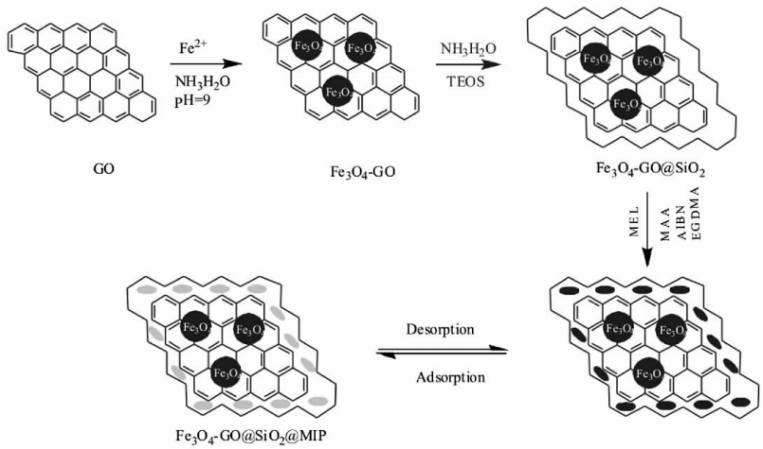


Fig. 1. Preparation procedure of magnetic melamine imprinted polymers

2.3. Static equilibrium adsorption experiment

A series of imprinted polymer (Fe3O4-GO@SiO2@MIP) and non-imprinted polymer (Fe3O4-GO@SiO2@NIP) are weighted accurately by 20mg respectively and be put into adsorption tube where 200mg/L MEL solution is added by 10mL respectively for static adsorption in 1h at room temperature. It is separated in the applied magnetic field after adsorption saturation and MEL concentration of supernatant in equilibrium liquid is measured with HPLC. Testing conditions of liquid chromatographic are shown as follows: testing wavelength λ=240nm; mobile phase is the ionic buffer solution and acetonitrile mixed solution (volume ratio of 9: 1); flow rate is 1.0mL/min; column temperature is 30°; injection volume is 10 L; ionic buffer solution is prepared with 10mmoL/L sodium 1-octanesulfonate and 10mmol/L citric acid (pH=3.0).

Polymer of Fe3O4-GO@SiO2@MIP and Fe3O4-GO@SiO2@NIP are weighted by 20mg respectively with adding 10mg/L MEL and competitive adsorption ((CYR and CYA) solution by 10mL respectively for static adsorption in 1h at room temperature. It is separated in the applied magnetic field after adsorption saturation and MEL and competitive adsorbate concentration of supernatant at equilibrium is measured with HPLC. Adsorbing capacity of polymer to various substances is calculated by the ratio of the deference between initial concentration and adsorption equilibrium concentration to the initial concentration.

2.4. Magnetic extraction experiment

Imprinted polymer (Fe3O4-GO@SiO2@MIP) is put into adsorption tube after weighted by 50mg accurately with adding 20mL labeled extraction solution of milk sample for static adsorption in 1h at room temperature. Fe3O4-GO@SiO2@MIP is separated in the applied magnetic field with solution discarded after adsorption saturation to be washed with 5.0mL of ethanol and eluted with 5.0mL of mixed solution (volume ratio of 9:1) of methanol and acetic acid. Then it is provided with magnetic separation, and the MEL in eluent is analyzed with HPLC.

3. Result and discussion

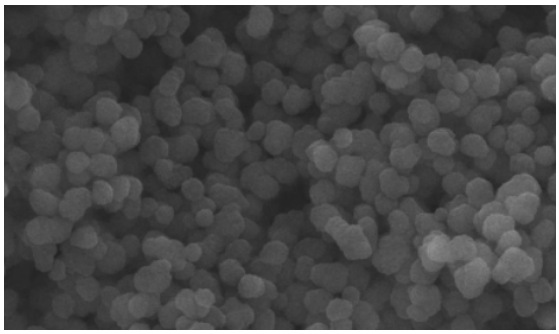
3.1. Preparation and structural characterization of imprinted polymer microspheres

The solvent has important effect on the level of adsorption property of prepared imprinted polymer microspheres. Common solvent and pore-foaming agent in process of molecular imprinting include chloroform, dichloromethane, acetonitrile, acetone and toluene and so on. Melamine belongs to alkalescent triazine heterocyclic compounds and its solubility in the common organic solvents is small. Chloroform, acetonitrile, acetone, toluene, methanol, ethylene glycol and mixed solvent of different proportion are tested for multiple times. The results indicate that solubility of melamine in the hot mixed solvents of ethylene glycol and acetonitrile is larger, which is able to meet experimental requirements. Acetonitrile and ethylene glycol (20: 1, V/V) are selected as the solvent and the pore-foaming agent in experiment, in the optimal volume of 40mL.

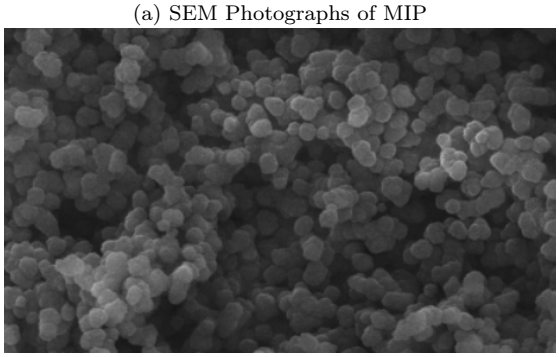
Precipitation polymer microspheres are formed in a large number of the reaction medium, precipitation polymerization is started in homogeneous-phase mixture. With the progress of reaction, oligomers are precipitated from the medium after crosslinked nucleation and mutually polymerize to form polymer particles. High crosslinked polymer microspheres are formed finally by capturing oligomers and functional monomers. It can be seen from Fig. 2 that the prepared MIP and N-MIP are in homogeneous shape, approximating to spherosome and the microspheres diameters distribution are $400 \sim 500$nm. However, particle size of imprinted polymer microspheres is larger than that of non-imprinted polymer microspheres, which is likely to be caused by that imprinted molecule occupies some space volumes and a large number of "holes" are left after elution so that they are represented as larger particle sizes in the case of basically same microspheres quantity.

3.2. Static equilibrium adsorption experiment

In this paper, GO is prepared with improved Hummers method and then magnetic GO is prepared on the surface of GO by modifying Fe3O4 in the alkaline conditions. A coat of SiO_2 is wrapped on the surface of magnetic GO with sol-gel method. Finally, imprinted polymer Fe3O4-GO@SiO2@MIP is prepared by grafted



(a) SEM Photographs of MIP

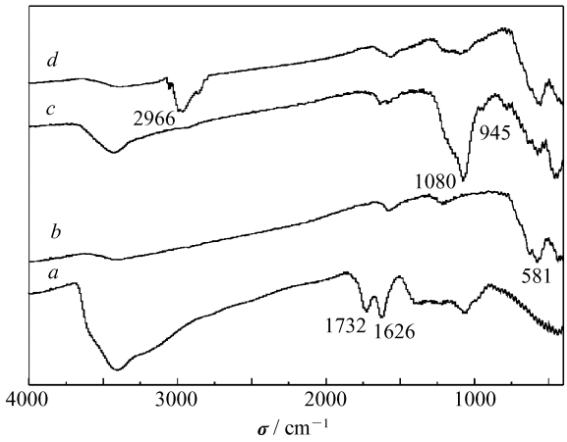


(b) SEM Photographs of N-MIP

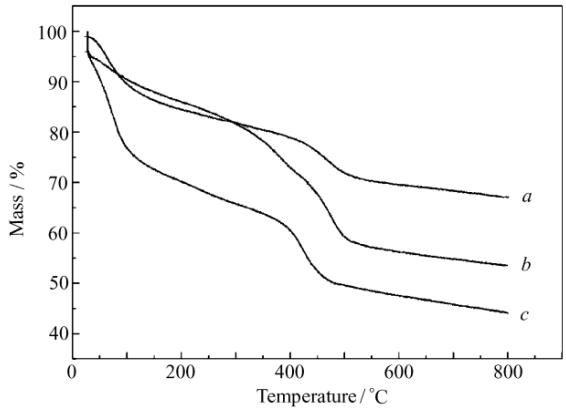
Fig. 2. SEM photographs

MEL imprinted shell on the surface of Fe3O4-GO@SiO2 with MAA as functional monomer, EGDMA as crosslinking agent and AIBN as initiator. Thickness of SiO_2 coat can be adjusted by changing proportion of TEOS and Fe3O4-GO. Research indicates that thickness of SiO_2 coat of the prepared Fe3O4-GO@SiO2 is just proper which will affect neither the stability of the imprinted polymer nor magnetic strength when V (TEOS): V (Fe3O4-GO) =2:1. Its morphology is characterized with SEM and TEM and the results are shown in Fig. 3 where the SEM photograph of GO prepared with the improved Hummers method is shown in Fig. 3 A, from which it can be seen that GO is the lamellar structure. SEM photograph of Fe3O4-GO is shown in Fig. 3B, from which it can be seen that Fe3O4 particles have been modified on the lamellar structure.

In order to research the affinity property of MIP, adsorption isotherms (as shown in Fig. 4) of solutions at different initial concentrations have been measured within 0.1∼1.5mmol/L melamine concentration. It can be known from the curves in Fig. 4 that adsorption capacity of molecularly imprinted polymer in unit mass is increased with growth of melamine concentration while adsorption capacity of N-MIP has become saturated when initial concentration of melamine is greater than 0.5mmol/L, and the adsorption capacity of MIP in unit mass is much greater than that of N-MIP in unit mass at the same initial concentration, which indicates that in the imprinting process, imprinted holes of template molecule produced by selective bonding in



(a) Infrared spectrum curve



(b) Thermogravimetric analysis curve

Fig. 3. Static equilibrium adsorption experiment

MIP and the active binding sites on the holes determine high affinity and specific recognition of MIP to template molecule much greater than non-selective bonding effect.

3.3. Magnetic property

Level of magnetic property is the key factor for the rapid separation of magnetic material in application. In order to investigate the magnetic property of such imprinted material, Fe3O4-GO@ SiO2 and Fe3 O4-GO@ SiO2 MIP are tested in their magnetic properties, with results as shown in Fig. 5. Saturation magnetizations of Fe3 O4-GO@ SiO2 and Fe3O4-GO@ SiO2@ MIP are 54.33 and 42.44 A·m2 /kg respectively. Magnetic strength of Fe3O4-GO@ SiO2MIP is slightly smaller than that of Fe3O4- GO@ SiO2, which is resulted from MIP coat on the Fe3O4-GO@ SiO2. Effective photograph of dispersion and magnetic separation process of Fe3 O4-GO@

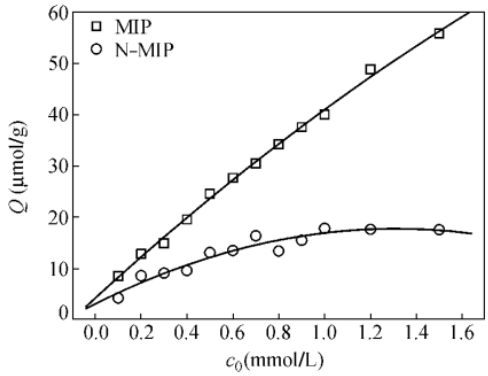


Fig. 4. Isotherm of melamine adsorption on MIP and N-MIP

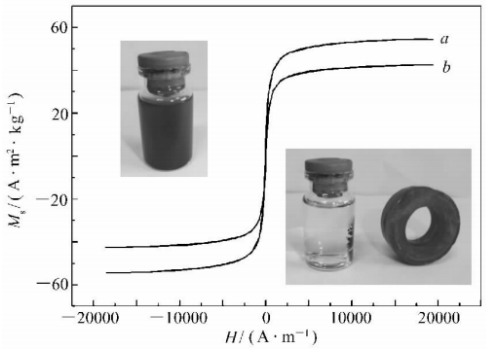


Fig. 5. Hysteresis loop

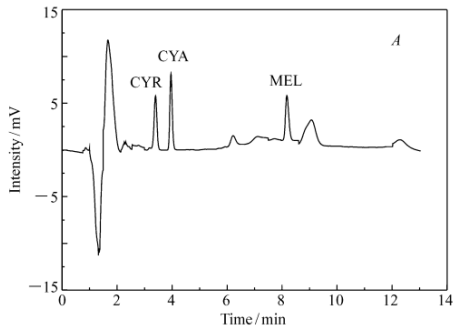
SiO2@ MIP is shown in Fig. 5. After ultrasonic dispersion, black magnetic imprinted polymer is dispersed in solution evenly. When external magnet is close to the bottle wall, Fe3 O4-GO@ SiO2 @MIP quickly moves towards the direction of the magnet and the mixed solution becomes clear, which indicates that the prepared Fe3 O4-GO@ SiO2 MIP has good magnetic property, applicable to M-SPE separation.

4. Application research

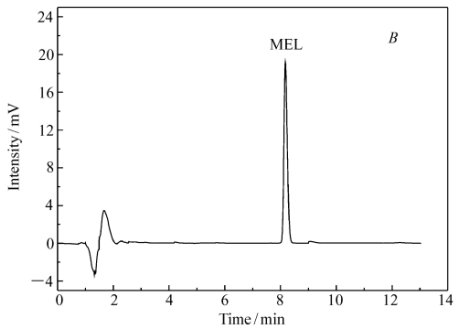
Mile samples are put into 10mL-scale centrifugal tube by 2.0g with adding a certain amount of standard solution and 40.0g/L sodium chloride solution by 4.0mL as well as phosphoric acid by 0.1mL for mixing. Then it is centrifuged at rate of 4000r/min in 10min and the supernatant is transferred into another 10mL centrifuge tube with adding 40.0g/L sodium chloride solution by 2.0mL. After above operations are repeated, the supernatant is merged for obtaining extraction of milk samples.

Since Fe3O4-GO@SiO2@MIP has the sufficient magnetic properties, it is applicable to M-SPE separation system. Combined with M-SPE and testing technology of HPLC, the separation and enrichment properties of MEL in milk samples are re-

searched. Labeled milk solution chromatogram is shown in Fig. 6 where labeled milk is washed with 5.0mL of ethanol firstly and then such magnetic imprinted polymer is eluted with 5.0mL of mixed solution (volume ratio of 9:1) of methanol and acetic acid. HPLC of the eluent is shown in Fig. 6B and other impurity peaks are decreased obviously while the chromatographic peak of MEL is increased significantly, which shows that use of such imprinted polymer is able to realize the separation and enrichment test of MEL in the sample solution.



(a) Sample solution of milk



(b) Solution after imprinted separation and enrichment

Fig. 6. Tested chromatography of MEL

5. Conclusion

Magnetic imprinted solid phase extraction material has been prepared on the surface of magnetic GO successfully with surface imprinting technology, which absorbs the melamine selectively. Testing of magnetic property indicates that such magnetic imprinted solid phase extraction material has excellent magnetic property and is applicable to M-SPE separation system. Specific surface area of the imprinted polymer is measured with N2 adsorption method to be 137. 1 m2 /g and the larger specific surface area promotes such magnetic imprinted solid phase extraction material has greater adsorption capacity to the template molecule. Results of absorption experiment show that a large number of effective binding sites exist in the imprinted

shell of the prepared magnetic MEL imprinted polymer, which is able to produce imprinted adsorption on MEL and quickly reach the adsorption equilibrium. Combined with solid phase extraction separation and enrichment technology and HPLC technology, the Fe3O4-GO@ SiO2@ MIP is used in enrichment and test for trace MEL in milk successfully. This technology has provided new method for the enrichment and determination of MEL in complex environment.

Acknowledgement

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